



Ultrasonographic diagnosis of twins in two pregnant bitches: case report

[Diagnóstico ultrassonográfico de gestação gemelar em duas cadelas: relato de caso]

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ABSTRACT

Multiple pregnancies in humans account for only 3% of pregnancies, 97-98% of which are twin pregnancies and the morbimortality is higher in the monochorionic twins when compared to dichorionic ones. The canine species is naturally multiparous, but the diagnosis of monochorionic twin pregnancy is not common. The objective of this report was to describe the ultrasonographic diagnosis of monochorionic twin pregnancies in two bitches [Pug (case 1) and Shih tzu (case 2)]. It was possible to verify the presence of one gestational vesicle containing two fetuses in each female by observing two heads or two bodies within the same placental site. These fetuses presented adequate viability and normal organogenesis. Their development was similar to the other fetuses. In case 1 they were stillborn and smaller than the other five live-born fetuses. The twins in case 2 were born alive, but they also appeared smaller when compared to the littermates. The gestational risks associated with this condition in pregnant bitches are still unknown, however, there are reports of fetal death in monochorionic pregnancies in this species. Therefore, ultrasonographic exam during pregnancy allows an early monochorionic diagnosis and monitoring the fetal viability could bring health benefits to both the female and the littermates.

Keywords: gemelar pregnancy, monochorionic, canine fetus

RESUMO

As gestações múltiplas em humanos correspondem a apenas 3% das gestações, sendo 97-98% dessas gestações gemelares. Sabe-se que a morbimortalidade fetal é maior em gêmeos monocoriônicos do que nos dicoriônicos. A espécie canina é naturalmente múltipara, mas o diagnóstico gestacional de gêmeos monocoriônicos não é comum. O objetivo deste relato é descrever o diagnóstico ultrassonográfico de gêmeos monocoriônicos em duas cadelas, sendo uma da raça Pug (caso 1) e outra da raça Shih-Tzu (caso 2). Foi possível verificar a presença de uma única vesícula gestacional contendo dois fetos em cada cadela, por meio da visibilização de duas cabeças ou de dois corpos dentro de uma mesma placenta. Esses fetos apresentavam viabilidade e organogênese adequadas e o grau de desenvolvimento era similar aos demais fetos da ninhada. No caso 1, os gêmeos nasceram mortos e de tamanho menor que os outros cinco fetos nascidos vivos. Os gêmeos do caso 2 nasceram vivos, mas também eram pequenos em relação aos irmãos da ninhada. Os riscos gestacionais associados a essa condição em cadelas ainda não são conhecidos, no entanto já existem relatos de morte fetal em gestação monocoriônica nessa espécie. Portanto, o exame ultrassonográfico durante a gestação permite um diagnóstico precoce da monocorionicidade, e a monitorização da viabilidade fetal pode trazer benefícios para a saúde da matriz, assim como para o restante da ninhada.

Palavras-chave: gestação gemelar, monocoriônico, feto canino

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INTRODUCTION

In the prenatal period, ultrasound is the best available tool to diagnose gestation, as well as to verify the viability and development of the litter since the embryonic period (Castro *et al.*, 2011). Early diagnosis occurs by visualization of gestational sacs at 10 to 20 days after luteinizing hormone (LH) peak in bitches. At that moment they are anechoic structures with a hyperechoic wall, which corresponds to the trophoblast. Between 23rd and 25th after the peak of LH, it is possible to identify the embryo, an elongated echogenic structure located within the gestational sac. A thin hyperechogenic layer internal to the uterus, corresponding to the developing placenta, surrounds it. The placenta becomes visible between 27 and 30 days after the LH peak and remains evident during organogenesis until the end of gestation (Mattoon and Nyland, 2015).

Multiple pregnancies in humans account for only 3% of pregnancies, 97-98% of which are twin pregnancies (Manso *et al.*, 2011). Twins are classified as dizygotic (when two eggs are fertilized by two spermatozoids) or monozygotic. In the second case, fertilization of a single ovum by one spermatozoid occurs with posterior division of the zygote. Depending on the moment of bipartition, the monozygotic twins can be dichorionic and diamniotics (complete and independent embryos, each one will have its own placenta and chorionic chamber), monochorionic and diamniotics (embryos share the same placenta and have a single chorion and separate amniotic chambers) or monochorionic and monoamniotics (single chorionic and amniotic chambers), different from the dizygotic ones that are always dichorionic and diamniotics (Silva *et al.*, 2003).

According to the results obtained by Manso *et al.* (2011), in women the morbimortality of multifetal pregnancy was superior to unifetal one, as well as it was higher in the monochorionic twins when compared to dichorionic ones. Therefore, an early ultrasound diagnosis, between the 11th and 13th week, is mandatory for adequate prenatal care to be instituted.

Bitches are naturally multiparous, so a meticulous ultrasonographic examination is necessary to access as many fetuses as possible.

Despite the numerous litters, the diagnosis of monochorionic twin pregnancy in this species is not common (Joonè *et al.*, 2016). The objective of this report was to describe the ultrasonographic diagnosis of monochorionic twin pregnancies in two bitches.

CASUISTRY

To confirm the pregnancy, two adult and healthy female dogs were submitted to image examination in the second trimester, using an ultrasound (Z6Vet, Mindray, China) with multifrequency 7.5MHz transducer, at a private veterinary clinic. One of the bitches was a 2-year-old Pug (case 1) and the other one was a Shih tzu (case 2).

During the ultrasonographic examination, an experienced operator confirmed gestation by the presence of fetal structures within the uterine silhouette. Uterus, placenta and amniotic fluid had no abnormalities. Adequate development and normal fetal heartbeats demonstrated gestational viability. It was possible to verify the presence of one gestational vesicle containing two fetuses in each female by observing two heads or two bodies within the same placental site, which indicated the presence of monochorionic twin pregnancy (Figures 1, 2). These fetuses presented adequate viability and normal organogenesis. Their development was similar to the other fetuses.

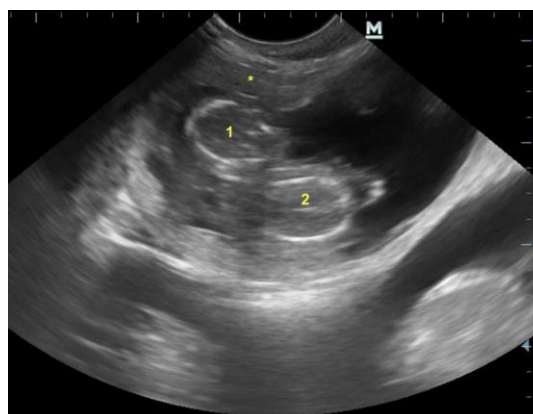


Figure 1. Ultrasonographic image of the skull of two distinct fetuses (1 and 2) sharing a single placental site (*) in a Pug bitch. The estimated gestational age was 38 days.



Figure 2. Ultrasonographic image of two concepts (1 and 2) sharing a single placental site (*) in a Shih tzu bitch. The estimated gestational age was 30 to 34 days.

Correlating ultrasonographic findings with the reproductive history, the gestational age in case 1 was approximately 38 days, when it was estimated the presence of six fetuses. After twenty days, a new evaluation was performed noting the absence of heartbeats in the twin fetuses. At night, they were stillborn, smaller than the other five live-born fetuses and they had their bodies covered by hair as expected for a full-term fetus. According to the owner, the twins presented no signs of maceration and they did not have any visible anomaly.

In case 2, gestational age was estimated from 30 to 34 days by using fetal echo biometry. It had an embryonic vesicle absorbed in left uterine horn. The heart rate of examined fetuses was within normal limits. The twins of this bitch were born alive, but they also appeared smaller when compared to the littermates. Both females had normal delivery.

DISCUSSION

The twins of cases 1 and 2 were visually smaller when compared to other canine neonates, similar to that observed in the report by Joonè *et al.* (2016), in which the monozygotic canine twins were born healthy, but significantly smaller than the other five of the litter.

Underdevelopment of twin fetuses, as well as cases of stillbirth, can be explained by the failure of a single placenta to supply the increased nutritional needs of two fetuses, which worsens in the third trimester of gestation when there is

rapid growth of fetuses in the dog (Joonè *et al.*, 2015). Fetal death of the pug bitch twins was detected exactly during this gestational period.

At approximately 58 days of gestation, ultrasonographic images of case 1 were compatible with fetal death for 1 or 2 days (the fetuses had no heart rate, the amniotic fluid was reduced and more echogenic). Similar to this case, Urhausen *et al.* (2017) evaluated the development of monozygotic twins at 38, 41, and 48 days post ovulation (p.o.) using ultrasound, but at 52 days p.o. they were dead. After cesarean section, the twins were found to be monozygotic and diamniotic and one of them had an anasarca. In addition, DNA exams of neonatal tissues have shown that they developed from two different oocytes.

Considering that there are reports of dizygotic canine fetuses sharing the same placental site, karyotyping genetic analysis of blood and tissues is the only method capable to confirm monozygosity (Joonè *et al.*, 2016). In the report described here, no test was performed to compare the DNA of the twins in either case. Therefore, it is not possible to affirm whether they were mono- or dizygotic and to determine whether the fetuses were mono- or diamniotic since the two bitches had normal deliveries and the owners had no experience to provide such information.

In the only report of genetically confirmed monozygotic twin dogs, the animals presented slight differences in pelage marks. For this reason, it is possible that these types of twins are not routinely identified in the litter, since dizygotic siblings in dogs may also be quite similar to each other (Joonè *et al.*, 2016). At this point, ultrasound examination appears as a tool capable of reducing the number of undiagnosed twin pregnancies.

Twin pregnancies are probably not diagnosed because many bitches do not undergo any type of examination as prenatal follow-up. An example is the case of Joonè *et al.* (2016), in which the female had her first gestational veterinary care when presenting dystocia. Urhausen *et al.* (2017) described the only ultrasonographic diagnosis of monozygotic twin gestation in dogs. In the other cases of twins sharing the same placental site this fact was only observed at birth (Duke,

1946, Joonè *et al.*, 2015, Joonè *et al.*, 2016, Nottidge *et al.*, 2007).

The absence of adequate gestational monitoring and the late recognition of dystocia leads to higher rates of neonatal mortality and health risks for the female (Johnson, 2008; Luz *et al.*, 2015). The tests performed to differentiate monozygotic and dizygotic twins are only possible after birth (Joonè *et al.*, 2016), therefore they do not contribute to the veterinarian management of the bitch. Thus, the present report shows ultrasonography as a relevant method from a practical point of view in the twinning diagnosis in the dog, since it was able to diagnose monozygosity in the two described cases at an early stage.

In humans, morbimortality in monozygotic pregnancies is three to four times higher than in dichorionic pregnancies. This is related to an increased risk of spontaneous abortion or fetal death before the 24th week, spontaneous preterm delivery, below-normal birth weight and structural malformations. In addition, in the event of one fetus' death, the other twin has a higher risk of perinatal death and neurological damage due to twin-twin transfusion syndrome, which occurs only in monozygosity events. Thus, the importance of an early diagnosis in addition to a cautious prenatal in cases of twinning pregnancy is already well established (Peralta and Barini, 2011).

Although the existence of these risks has not been defined in the dog, similar situations to what is reported in humans were described in the few cases described before and in the present report, as below average weight, malformations and fetal death (Joonè *et al.*, 2016, Nottidge *et al.*, 2007, Urhausen *et al.*, 2017). Therefore, the ultrasound diagnosis is fundamental to guide the conduct of the veterinarian, such as suggesting a protocol of periodic exams during gestational follow-up, in order to guarantee delivery assistance and perform a cesarean section if necessary.

CONCLUSION

The presence of two fetuses sharing the same placental site on ultrasonography exam allows the diagnosis of monozygotic twins in canine species. The gestational risks associated with this

condition in pregnant bitches are still unknown, however, there are reports of fetal death in monozygotic pregnancies in this species. Therefore, ultrasonographic exam during pregnancy allows an early monozygosity diagnosis and monitoring the fetal viability could bring health benefits to both female and the littermates.

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